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# EVALUATION OF FUNGAL POPULATIONS ON PONDEROSA PINE SEED

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by

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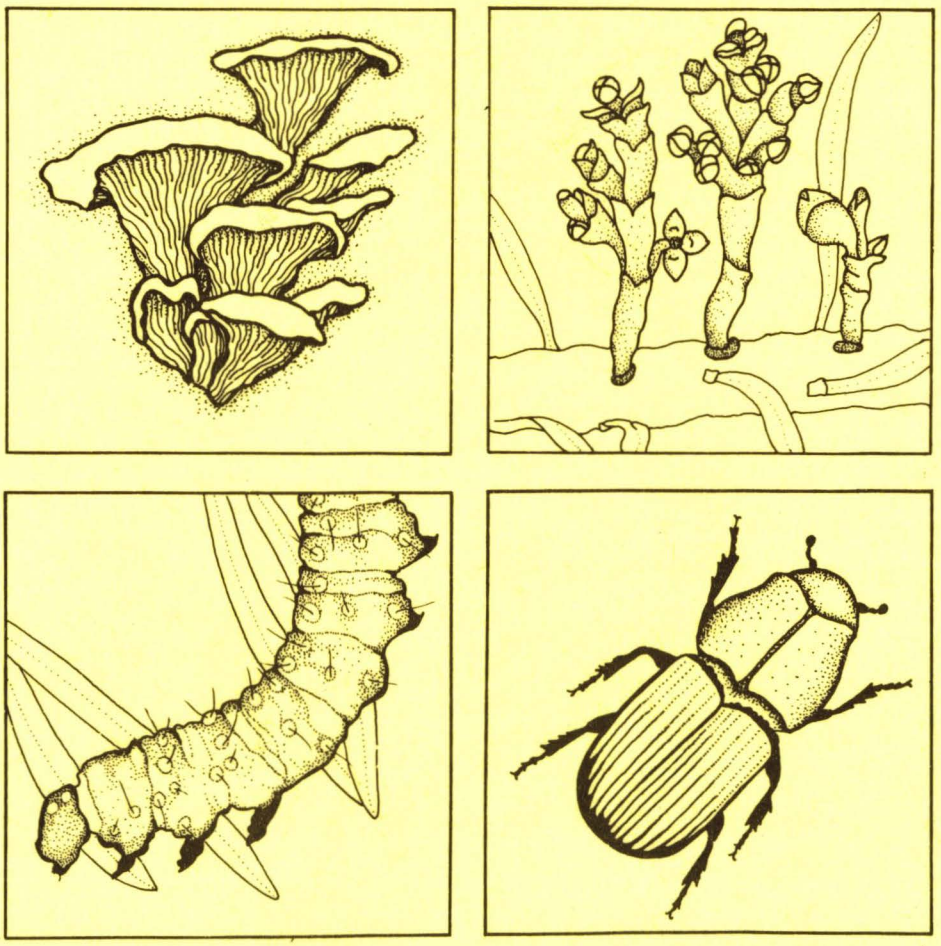

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# EVALUATION OF FUNGAL POPULATIONS ON PONDEROSA PINE SEED

by

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## ABSTRACT

Abundance and identity of fungi colonizing ponderosa pine seed were determined on eight selected seedlots collected for the Champion Timberlands Nursery, Plains, MT. Thirty groups of organisms were found within the seedlots; surface sterilization with Clorox® did not remove all fungi from seed. Although potential of isolated organisms to cause diseases of seed and seedlings is unknown, several contaminating fungi including Fusarium, Pythium, Botrytis, and Verticillium are possible pathogens. Seed treatment to reduce or eliminate fungal contamination is necessary to produce healthy seedlings at the nursery.

## INTRODUCTION

Many different fungi commonly colonize seed from conifer trees. Although most of these organisms do not adversely affect seed, some may be pathogens and reduce germination or initiate disease on young seedlings. Many common seed colonizers are ubiquitous in distribution (Neergaard 1977). Abundance of potential pathogenic fungi on seed is often related to seed source (Anderson et al. 1980; Mason and Van Arsdel 1978). For example, seed collected from squirrel caches might be more contaminated with common soilborne pathogens, whereas contamination of seed collected directly from trees should be less (Sutherland 1979).

Problems with contaminated ponderosa pine (Pinus ponderosa Laws.) seed at the Champion Timberlands Nursery, Plains, MT have been previously reported (James and Genz 1981). Contaminated seed generally displayed reduced germinability and emerging seedlings often became diseased, presumably from seedborne organisms. The most promising seed treatment tested so far involved soaking seed in 0.03 percent hydrogen peroxide for 5 hours followed by a thorough rinsing with tap water. However, even such treatment does not always remove all contaminating fungi (James and Genz 1981).

A continuing program is underway to improve the quality and vigor of seedlings produced at the nursery. Providing a continuous supply of disease-free seed is an important initial step in this program. As part of this program, an evaluation was conducted to identify fungi commonly colonizing ponderosa pine seed and to determine their relative abundance within several different seedlots. We also sought information on their ecological roles on and within seed.

## MATERIALS AND METHODS

Seed for this evaluation was collected from eight sources on land owned by Champion Timberlands (table 1). Five sources were from squirrel caches; the remainder were from recently felled trees. Seed sources were typical of those commonly used by the nursery.

Table 1.--Descriptions of ponderosa pine seedlots.

| Seedlot number | Description |
|---|---|
| 1 | Obtained near Herrin Lakes (Sec. 1, T13N, R9W), 9/1/81 at about 4,900 ft. elevation. Collected from felled trees. No apparent insect damage or mold on cones. |
| 2 | Obtained near Herrin Lakes (Sec. 1, T13N, R9W), 9/14/81 at about 4,800 ft. elevation. Collected from felled trees. No apparent insect damage or mold on cones. |
| 3 | Obtained near Doney Meadows (Sec. 14, T15N, R12W), 9/1/81 at about 4,500 ft. elevation. Collected from felled trees. No apparent insect damage or mold on cones. |
| 4 | Obtained near Hay Creek (Sec. 9, T14N, R24W), 9/6/81 at about 4,200 ft. elevation. Collected from squirrel caches. No apparent insect damage or mold on cones. |
| 5 | Obtained near Bear Springs (Sec. 7, T27N, R25W), 9/8/81 at about 3,800 ft. elevation. Collected from squirrel cache. Some insect damage was present; no apparent mold was on cones. |
| 6 | Obtained near East Twin (Sec. 23, T14N, R17W), 9/16/81 at about 4,800 ft. elevation. Collected from squirrel cache. Some insect damage was present; no apparent mold was on cones. |
| 7 | Obtained near Two Creeks (Sec. 4, T16N, R12W), 9/17/81 at about 4,200 ft. elevation. Collected from squirrel cache. No insect damage was apparent; light mold was found on cones. |
| 8 | Obtained near Indian Creek (Sec. 16, T25N, R27W), 9/14/81 at about 3,600 ft. elevation. Collected from squirrel cache and standing trees. Some insect damage was present; no apparent mold was on cones. |

Seed was extracted after kiln drying the cones at 38-94°C for 45-96 hours. Seed was scalped, dewinged, and placed in an air separator to remove light, nonviable seed. Seed was then stratified for 30 days at about 0-2°C. Following stratification, seed was placed in plastic bags and kept refrigerated (15°C) prior to laboratory analysis.

We suspected that fungi commonly found externally on seedcoats would be removed by rinsing in water or surface sterilization with 10 percent aqueous sodium hypochlorite. Most fungi located within seed could probably be determined if seeds were aseptically dissected and incubated with endosperms exposed. Therefore, these treatments were conducted (table 2).

After treatment, seed was aseptically placed on 2 percent water agar in 9 cm petri plates and incubated at 24°C under white fluorescent lights. Seed was checked periodically for fungal colonization after 5 days' incubation. One hundred seeds were evaluated per seedlot (25 for each of four treatments).

Table 2.--Description of ponderosa pine seed treatments.

| Treatment number | Description |
|---|---|
| 1 | Seed placed directly on 2 percent water agar without washing (no treatment-check). |
| 2 | Seed placed in a continuous tap water bath, agitated by the running water stream. Duration of the wash was 1 hour. Washed seed was then aseptically placed on 2 percent water agar. |
| 3 | Seed surface sterilized in 10 percent sodium hypochlorite (Clorox®) for 10 minutes, then rinsed three times with sterile water and aseptically placed on 2 percent water agar. |
| 4 | Seeds were treated as in #3 above, but were dissected in half aseptically with a sterile scalpel and placed on 2 percent water agar with the exposed endosperm surface up. |

Fungi colonizing seed were initially classified by group based on gross (10x) morphological characteristics. Occurrence of fungal groups among seedlots by treatment was tallied. Each group was subcultured onto potato dextrose agar slants and identified to genus using several standard taxonomic guides (Barnett and Hunter 1972; Clements and Shear 1957; Kendrick and Carmichael 1973). Speciation was determined for selected genera using several monographs (Booth 1971; Ellis 1971; Kulick 1968; Middleton 1943; Raper and Thom 1949; Simmons 1967; Toussoun and Nelson 1968).

Seeds were also checked periodically for germination after 13 days' incubation. A final check for germination was made after 1 month; we felt that little germination would occur thereafter because of extensive fungal contaminations.

All ungerminated seeds were dissected to determine condition of the endosperm after incubation for 1 month. Discolored, decayed, or milky endosperms which lacked firmness were considered diseased. Fungi colonizing diseased tissues were noted if they were sporulating; if not, diseased tissues were cultured on 2 percent water agar and emerging fungi identified.

Hypocotyls of germinated seed were checked periodically for disease, indicated by darkened lesions, water soaking, and fungal colonization associated with tissue necrosis. Associated fungi were identified if they were sporulating; if not, sections of diseased hypocotyls were cultured on 2 percent water agar and emerging fungi identified.

Occurrence of fungi on seed, diseased endosperms, and hypocotyls was compared by seed source and treatment with an analysis of variance. For those fungi with significant differences (P=0.01 or P=0.05), colonization of seed from different seed sources or undergoing different treatments was compared using Duncan's Multiple Range Comparison Test.

## RESULTS

Twenty-nine different groups of fungi were identified on ponderosa pine seed (tables 3 and 4). All bacteria colonizing seed were grouped together and identification was not attempted. The major fungi found colonizing seed included several species of Penicillium (primarily P. chrysogenum Thom, P. claviforme Bainier, P. glabrum (Wehner) Westling, P. oxalicum Currie and Thom, and P. viridicatum Westling), Aureobasidium pullulans (DeBary) Arnauld, Cladosporium cucumerinum Ellis and Arth., two species of Fusarium (F. oxysporum Schlecht. and F. solani (Mart.) Sacc.), Mucor mucedo L., Pythium aphanidermatum (Eds.) Fitz., Rhizopus arrhizas Fischer, Trichoderma viride Pers. and species of Ulocladium and Verticillium. Several other fungi were less common on seed.

Table 3.--Occurrence of fungi on ponderosa pine seedlots from the Champion Timberlands Nursery, Plains, MT.

| Fungus | Occurrence within seedlot: Number of seedlots | Occurrence within seedlot: Percent | Number of seed colonized per seedlot [1/]: 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | F Value [2/] |
|---|---|---|---|---|---|---|---|---|---|---|---|
| *Alternaria alternata* | 3 | 37.5 | 0 | 1 | 0 | 0 | 0 | 2 | 0 | 2 | 0.7 [5/] |
| *Aspergillus* sp. | 1 | 12.5 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | - [6/] |
| *Aureobasidium pullulans* | 8 | 100.0 | 8 | 10 | 11 | 4 | 14 | 10 | 19 | 11 | 1.5 [5/] |
| Bacteria (unidentified) | 8 | 100.0 | 10 | 8 | 9 | 2 | 10 | 6 | 6 | 11 | 1.0 [5/] |
| *Botrytis cinerea* | 2 | 25.0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | - [6/] |
| *Cephalosporium* sp. | 1 | 12.5 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | - [6/] |
| *Chaetomium* sp. | 3 | 37.5 | 0 | 0 | 0 | 5 | 3 | 0 | 0 | 3 | 1.0 [5/] |
| *Cladosporium cucumerinum* | 7 | 87.5 | 1 | 4 | 6 | 4 | 4 | 0 | 4 | 3 | 0.9 [5/] |
| *Diplodia pinea* | 1 | 12.5 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | - [6/] |
| *Fusarium oxysporum* | 6 | 75.0 | 0 | 8 | 2 | 3 | 0 | 2 | 4 | 1 | 1.9 [5/] |
| *Fusarium solani* | 2 | 25.0 | 0 | 0 | 3 | 1 | 0 | 0 | 0 | 0 | - [6/] |
| *Gliocladium* sp. | 3 | 37.5 | 0 | 0 | 1 | 0 | 0 | 0 | 9 | 2 | 0.9 [5/] |
| *Lacellina graminicola* | 1 | 12.5 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | - [6/] |
| *Mucor mucedo* * | 8 | 100.0 | 29C | 31C | 29C | 50A | 31C | 41B | 53A | 47AB | 5.8 [3/] |

Table 3.--continued.

| Fungus | Occurrence within seedlot: Number of seedlots | Occurrence within seedlot: Percent | Number of seed colonized per seedlot [1]: 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | F: Value [2] |
|---|---|---|---|---|---|---|---|---|---|---|---|
| *Penicillium chrysogenum* * | 8 | 100.0 | 54A | 49AB | 41B | 31C | 52A | 41B | 56A | 67A | 5.6 [3] |
| *Penicillium claviforme* | 7 | 87.5 | 16 | 0 | 13 | 5 | 9 | 26 | 15 | 11 | 1.8 [5] |
| *Penicillium expansum* | 1 | 12.5 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | - [6] |
| *Penicillium fuscum* | 1 | 12.5 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | - [6] |
| *Penicillium glabrum* * | 8 | 100.0 | 45BC | 38C | 77A | 57B | 54B | 60AB | 56B | 56B | 8.9 [3] |
| *Penicillium oxalicum* * | 8 | 100.0 | 16B | 16B | 34A | 13B | 22AB | 17B | 26A | 18B | 4.1 [3] |
| *Penicillium viridicatum* | 8 | 100.0 | 6 | 5 | 7 | 7 | 2 | 10 | 2 | 10 | 1.1 [5] |
| *Phoma* sp. | 1 | 12.5 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | - [6] |
| *Pyrenochaeta* sp. | 3 | 37.5 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | - [6] |
| *Pythium aphanidermatum* | 6 | 75.0 | 0 | 3 | 1 | 3 | 0 | 2 | 1 | 2 | 0.7 [5] |
| *Rhizopus arrhizas* * | 6 | 75.0 | 46AB | 2D | 24BC | 65A | 9CD | 6D | 0D | 0D | 20.3 [3] |
| *Trichoderma viride* * | 8 | 100.0 | 3C | 9BC | 7C | 24AB | 17B | 32A | 12B | 23AB | 3.3 [3] |
| *Trichothecium roseum* | 4 | 50.0 | 0 | 0 | 0 | 5 | 1 | 1 | 2 | 0 | 1.0 [5] |

Table 3.--continued.

| Fungus | Occurrence within seedlot: Number of seedlots | Occurrence within seedlot: Percent | Number of seed colonized per seedlot 1/: 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | F Value 2/ |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Ulocladium sp. * | 2 | 25.0 | 0B | 0B | 0B | 1B | 7A | 0B | 0B | 0B | 2.1 4/ |
| Verticillium sp. * | 3 | 37.5 | 0C | 0C | 0C | 0C | 0C | 16A | 9B | 7B | 4.5 3/ |
| Yeast (Unidentified) | 2 | 25.0 | 3 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | - 6/ |

1/ Maximum number of seed per seedlot is 100. Seedlots described in table 1.

2/ F values from an analysis of variance comparing fungal colonization among seedlots.

3/ Statistically significant (P=0.01).

4/ Statistically significant (P=0.05).

5/ Not statistically significant.

6/ Not calculated because of low occurrence of the fungus.

* Fungi with asterisk have significant F values (P=0.01 or P= 0.05); within each row for these fungi, number of seed colonized followed by the same capital letter are not statistically different (P=0.05) among seedlots using Duncan's Multiple Range Comparison Test.

Table 4.--Effects of treatments on the occurrence of fungi on ponderosa pine seed from the Champion Timberlands Nursery, Plains, Montana.

| Fungus | Colonization of Seed | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | All treatments [1] | | Treatment 1 | | Treatment 2 | | Treatment 3 | | Treatment 4 | | |
| | No. | Percent | No. | Percent | No. | Percent | No. | Percent | No. | Percent | F Value [2] |
| *Alternaria alternata* | 5 | 0.6 | 2 | 1.0 | 2 | 1.0 | 1 | 0.5 | 0 | 0 | 1.2 [5] |
| *Aspergillus* sp. | 2 | 0.3 | 2 | 1.0 | 0 | 0 | 0 | 0 | 0 | 0 | - [6] |
| *Aureobasidium pullulans* * | 87 | 10.9 | 5C | 2.5 | 18B | 9.0 | 44A | 22.0 | 20B | 10.0 | 10.7 [3] |
| Bacteria (Unidentified) | 58 | 7.3 | 14 | 7.0 | 12 | 6.0 | 8 | 4.0 | 24 | 12.0 | 1.4 [5] |
| *Botrytis cinerea* | 2 | 0.3 | 1 | 0.5 | 1 | 0.5 | 0 | 0 | 0 | 0 | - [6] |
| *Cephalosporium* sp. | 1 | 0.1 | 0 | 0 | 1 | 0.5 | 0 | 0 | 0 | 0 | - [6] |
| *Chaetomium* sp. | 11 | 1.4 | 2 | 1.0 | 0 | 0 | 9 | 4.5 | 0 | 0 | 1.0 [5] |
| *Cladosporium cucumerinum* | 26 | 3.3 | 5 | 2.5 | 5 | 2.5 | 2 | 1.0 | 14 | 7.0 | 0.1 [5] |
| *Diplodia pinea* | 1 | 0.1 | 0 | 0 | 0 | 0 | 1 | 0.5 | 0 | 0 | - [6] |
| *Fusarium oxysporum* | 20 | 2.5 | 8 | 4.0 | 2 | 1.0 | 4 | 2.0 | 6 | 3.0 | 1.9 [5] |
| *Fusarium solani* | 4 | 0.5 | 1 | 0.5 | 0 | 0 | 0 | 0 | 3 | 1.5 | - [6] |
| *Gliocladium* sp. | 12 | 1.5 | 7 | 3.5 | 5 | 2.5 | 0 | 0 | 0 | 0 | 2.0 [5] |
| *Lacellina graminicola* | 2 | 0.3 | 2 | 1.0 | 0 | 0 | 0 | 0 | 0 | 0 | - [6] |

Table 4.--continued.

| Fungus | Colonization of Seed | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | All treatments [1] | | Treatment 1 | | Treatment 2 | | Treatment 3 | | Treatment 4 | | |
| | No. | Percent | No. | Percent | No. | Percent | No. | Percent | No. | Percent | F Value [2] |
| Mucor mucedo * | 311 | 38.9 | 189A | 94.5 | 79B | 39.5 | 28C | 14.0 | 15D | 7.5 | 262.0 [8] |
| Penicillium chrysogenum * | 391 | 48.9 | 169A | 84.5 | 155B | 77.5 | 45C | 22.5 | 22C | 11.0 | 214.9 [3] |
| Penicillium claviforme * | 95 | 11.9 | 17B | 8.5 | 40A | 20.0 | 22B | 11.0 | 16B | 8.0 | 10.1 [3] |
| Penicillium expansum | 2 | 0.3 | 0 | 0 | 0 | 0 | 2 | 1.0 | 0 | 0 | - [6] |
| Penicillium fuscum | 1 | 0.1 | 0 | 0 | 1 | 0.5 | 0 | 0 | 0 | 0 | - [6] |
| Penicillium glabrum * | 437 | 54.6 | 177A | 88.5 | 160B | 80.0 | 80C | 40.0 | 20D | 10.0 | 319.3 [3] |
| Penicillium oxalicum * | 210 | 26.3 | 91A | 45.5 | 76B | 38.0 | 24C | 12.0 | 19C | 9.5 | 36.4 [3] |
| Penicillium viridicatum | 49 | 6.1 | 11 | 5.5 | 23 | 11.5 | 9 | 4.5 | 6 | 3.0 | 4.2 [5] |
| Phoma sp. | 1 | 0.1 | 1 | 0.5 | 0 | 0 | 0 | 0 | 0 | 0 | - [6] |
| Pyrenochaeta sp. | 3 | 0.4 | 1 | 0.5 | 1 | 0.5 | 1 | 0.5 | 0 | 0 | - [6] |
| Pythium aphanidermatum | 12 | 1.5 | 0 | 0 | 0 | 0 | 5 | 2.5 | 7 | 3.5 | 1.2 [5] |
| Rhizopus arrhizas * | 152 | 19.0 | 63A | 31.5 | 46B | 23.0 | 17C | 8.5 | 26C | 13.0 | 12.7 [3] |

Table 4.--continued.

| Fungus | Colonization of Seed | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | All treatments 1/ | | Treatment 1 | | Treatment 2 | | Treatment 3 | | Treatment 4 | | |
| | No. | Percent | No. | Percent | No. | Percent | No. | Percent | No. | Percent | F Value 2/ |
| Trichoderma viride * | 123 | 15.4 | 76A | 38.0 | 36B | 18.0 | 8C | 4.0 | 3C | 1.5 | 10.8 3/ |
| Trichothecium roseum | 9 | 1.1 | 3 | 1.5 | 6 | 3.0 | 0 | 0 | 0 | 0 | 1.0 5/ |
| Ulocladium sp. | 8 | 1.0 | 1 | 0.5 | 1 | 0.5 | 1 | 0.5 | 5 | 2.5 | 0.7 5/ |
| Verticillium sp.* | 32 | 4.0 | 28A | 14.0 | 4B | 2.0 | 0B | 0 | 0B | 0 | 10.4 3/ |
| Yeast (Unidentified) | 4 | 0.5 | 1 | 0.5 | 2 | 1.0 | 1 | 0.5 | 0 | 0 | - 6/ |



1/ Treatments are described in table 2.

2/ F values from an analysis of variance comparing fungal colonization among treatments.

3/ Statistically significant (P=0.01).

4/ Statistically significant (P=0.05).

5/ Not statistically significant.

6/ Not calculated because of low occurrence of the fungus.

* Fungi with asterisk have significant F values (P=0.01 or P=0.05); within each row for these fungi, number of seed colonized followed by the same capital letter are not statistically different (P=0.05) among treatments using the Duncan's Multiple Range Comparison Test.

Most colonizing fungi were well distributed within all eight seedlots. Although several statistical differences were found in colonization among seedlots (table 3), importance of these differences with regard to disease of seed or emerging hypocotyls is unknown, because pathogenic potential of these fungi is unclear.

Many different fungi were isolated from diseased endosperms and hypocotyls (table 5). Some fungi commonly considered pathogens, such as F. oxysporum, F. solani, P. aphanidermatum, and Verticillium spp. were more commonly isolated from diseased tissues than externally on seedcoats. This may indicate that these fungi are more commonly carried within seed or their abundance on seedcoats may be limited by competition with the many other fungi colonizing this substrate.

Washing and treating seed with a surface sterilant reduced abundance of many fungi on seedcoats (table 4). Colonization by most Penicillium spp. and other common seed fungi such as M. mucedo and R. arrhizas decreased following seed treatment. On the other hand, A. pullulans and Chaetomium sp. were more commonly isolated from treated seed. These organisms may have been more resistant to treatments and more apparent because of reductions in other competing seed fungi. Treatments used in this evaluation did not result in "clean" seed free from fungal contamination. However, the surface sterilization treatment (treatment 3) reduced abundance of many fungi.

Overall germination of the ponderosa pine seed tested in this evaluation was poor; percentages ranged from 72 percent (seedlot 2) to about 15 percent (seedlots 4 and 5) (table 6).

We believe that much of the seed was injured during dewinging. Evidence of this was confirmed by X-rays of selected seed from the eight seedlots which revealed damaged endosperms. Another possible problem was that the high temperatures used to dry cones may have damaged seed. Of the seed that did germinate, almost 35 percent had diseased hypocotyls during the period for which this evaluation was made. We believe that fungi carried on or within seedcoats were responsible for this hypocotyl colonization.

About 25 percent of the nongerminated seed was diseased when dissected and examined (table 6). Despite efforts to remove empty seed prior to the evaluation, about 10 percent of the ungerminated seed lacked endosperms.

Table 5.--Abundance of fungi on diseased endosperms and hypocotyls of ponderosa pine seed from the Champion Timberlands Nursery, Plains, Montana. 1/

| Fungus | Colonization of diseased endosperms | | Colonization of diseased hypocotyls | |
|---|---|---|---|---|
| | Number | Percent | Number | Percent |
| Alternaria alternata | 1 | 0.5 | 1 | 1.5 |
| Aureobasidium pullulans | 23 | 11.5 | 2 | 3.0 |
| Bacteria (unidentified) | 11 | 5.5 | 0 | 0 |
| Botrytis cinerea | 1 | 0.5 | 0 | 0 |
| Chaetodumium sp. | 10 | 5.0 | 0 | 0 |
| Cladosporium cucumerinum | 16 | 8.0 | 2 | 3.0 |
| Diplodia pinea | 1 | 0.5 | 0 | 0 |
| Fusarium oxysporum | 12 | 6.0 | 7 | 10.4 |
| Fusarium solani | 6 | 3.0 | 0 | 0 |
| Gliocladium sp. | 7 | 3.5 | 2 | 3.0 |
| Mucor mucedo | 38 | 19.0 | 9 | 13.4 |
| Penicillium chrysogenum | 12 | 6.0 | 6 | 8.9 |
| Penicillium claviforme | 65 | 32.5 | 13 | 19.4 |
| Penicillium expansum | 1 | 0.5 | 1 | 1.5 |
| Penicillium fuscum | 1 | 0.5 | 0 | 0 |
| Penicillium glabrum | 5 | 2.5 | 5 | 7.5 |
| Penicillium oxalicum | 88 | 44.0 | 10 | 14.9 |

Table 5. continued

| Fungus | Colonization of diseased endosperms | | Colonization of diseased hypocotyls | |
|---|---|---|---|---|
| | Number | Percent | Number | Percent |
| Penicillium viridicatum | 7 | 3.5 | 0 | 0 |
| Pyrenochaeta sp. | 1 | 0.5 | 0 | 0 |
| Pythium aphanidermatum | 10 | 5.0 | 0 | 0 |
| Rhizopus arrhizas | 7 | 3.5 | 2 | 3.0 |
| Trichoderma viride | 66 | 33.0 | 16 | 23.9 |
| Trichothecium roseum | 8 | 4.0 | 0 | 0 |
| Ulocladium sp. | 2 | 1.0 | 0 | 0 |
| Verticillium sp. | 11 | 5.5 | 3 | 4.5 |

1/ Diseased endosperms and hypocotyls have discoloration, decay, and lack firmness. Fungi described were colonizing diseased tissues.

Table 6.--Incidence of disease on germinated and nongerminated ponderosa pine seed from the Champion Timberlands Nursery, Plains, Montana. 1/

| Seedlot | Germinated seed | | | Nongerminated seed | | | |
|---|---|---|---|---|---|---|---|
| | All seed | Diseased | Nondiseased | All seed | Diseased | Nondiseased | Empty |
| 1 | 24 (32.0) | 9 (37.5) | 15 (62.5) | 51 (68.0) | 9 (17.6) | 36 (70.6) | 6 (11.8) |
| 2 | 54 (72.0) | 13 (24.1) | 41 (75.9) | 21 (28.0) | 5 (23.8) | 8 (38.1) | 8 (38.1) |
| 3 | 20 (26.7) | 8 (40.0) | 14 (60.0) | 55 (73.3) | 25 (45.4) | 14 (25.4) | 16 (29.2) |
| 4 | 11 (14.7) | 2 (18.2) | 9 (81.8) | 64 (85.3) | 7 (10.9) | 52 (81.2) | 5 ( 7.9) |
| 5 | 11 (14.7) | 5 (45.4) | 6 (54.6) | 64 (85.3) | 17 (26.6) | 46 (71.9) | 1 ( 1.5) |
| 6 | 27 (36.0) | 12 (44.4) | 15 (55.6) | 48 (64.0) | 17 (35.4) | 31 (64.6) | 0 ( 0 ) |
| 7 | 30 (40.0 | 10 (33.3) | 20 (67.7) | 45 (60.0) | 11 (24.4) | 32 (71.1) | 2 ( 4.5) |
| 8 | 16 (21.3) | 8 (50.0) | 8 (50.0) | 59 (78.7) | 13 (22.0) | 42 (71.2) | 4 ( 6.8) |
| Totals | 193 (32.2) | 67 (34.7) | 126 (65.3) | 407 (67.8) | 104 (25.5) | 261 (64.1) | 42 (10.4) |

1/ Diseased seed were those with discoloration, decay, and lack of endosperm firmness. Diseased germinated seed were those with similar discoloration and/or fungal colonization on the emerging hypocotyl. Values in the table are number of seed with percentages in parentheses. Seed from all treatments except #4 (dissected seed) were included in the data.

## DISCUSSION

Many different groups of fungi were common inhabitors of ponderosa pine seed. Our data did not reveal striking differences in fungal populations on seed from squirrel caches and recently felled trees, although previous work (Sutherland 1979) had shown that occurrence of certain pathogenic fungi was more common on seed from squirrel caches.

The most common group of seed colonizers was from the genus Penicillium. These fungi are very common colonizers of soil and organic debris (Raper and Thom 1949; Smith 1963). Several species are common on seed of conifers (Edwards and Sutherland 1979; James and Genz 1981; Mason and Van Arsdel 1978) and hardwood forest trees (Janerette 1979). The major species we encountered were P. chrysogenum, P. oxalicum, P. glabrum, P. viridicatum, and P. claviforme. Penicillium chryosgenum was previously reported as causing rot of maize seed (Noble and Richardson 1968). Penicillium oxalicum is a common rhizosphere inhabitant (Windels and Kommendahl 1982) and has been used as a seed protectant against soil pathogens such as Fusarium spp. and Pythium spp. (Windels and Kommendahl 1978). However, P. oxalicum also occurs as a storage rot organism on maize (Noble and Richardson 1968) and can cause yellowing of maize leaves (Johann et al. 1931). The other Penicillium species we found on ponderosa pine seed are generally less common in nature and usually act as saprophytes on dead organic matter (Neergaard 1977; Raper and Thom 1949).

Two other groups of ubiquitous mold fungi were common on pine seed. The first group consisted of the common black molds Rhizopus arrhizas and Mucor mucedo. Rhizopus occurs on many different substrates, such as decaying organic matter in the soil and necrotic plant tissues (Alexopoulous 1962; Neergaard 1977; Stevens 1974). The fungus may also infect and decay cotton seed (Halisky and Satour 1964). Mucor also frequently colonizes different substrates (Stevens 1974) and may contribute to the deterioration of oak and beech seeds. Apparently the fungus is soilborne and attacks seed on the ground (Neergaard 1977). The other group of common molds we isolated from pine seed included Trichoderma viride and Aureobasidium pullulans. Trichoderma frequently colonizes soil organic matter; it often produces strong antibiotics and is sometimes parasitic on other fungi (Dennis and Webster 1971; Neergaard 1977). Aureobasidium is usually found on necrotic plant tissues or organic matter within the soil (Neergaard 1977). It may also be carried within seed (Pugh and Buckley 1971) and is considered a weak pathogen at times (Cooke 1959).

Several of the fungi we isolated from pine seed are often considered plant pathogens. Cladosporium cucumerinum may be seedborne and cause deterioration of vegetable seed (Malone and Muskett 1964; Neergaard 1977). The fungus also causes disease on several vine crops (Sherf 1964), peas (Snyder 1934), and the foliage of forest trees (Campana and

Rosinski 1962; Hepting 1971). Another possible pathogen, Alternaria alternata, is a common epiphyte on many plants (Hepting 1971) and may colonize the seed of numerous crops (Neergaard 1977), including forest trees (Mason and Van Arsdel 1978). The fungus is also capable of parasitizing Jack pine seedlings (Vaartaja and Cram 1956). We also isolated two species of plant pathogenic Fusarium. The first of these, F. oxysporum, is a common pathogen in conifer nurseries (Sutherland and Van Eerden 1980) and is often found contaminating the seed of many different plants (Neergaard 1977). As a seed contaminant, it initially exists as a saprophyte externally on the seedcoat, then may invade the seed. The other Fusarium isolated (F. solani) is a frequent pathogen that may also occur on plant seeds (Neergaard 1977; Noble and Richardson 1968).

Other possible pathogens isolated from pine seed include Pythium aphanidermatum, Botrytis cinerea, Diplodia pinea, and Verticillium sp. Pythium is a soilborne fungus that often causes disease in nursery seedbeds (James 1982). It has also been reported as a seed pathogen of watermelon, cucumber, and tomato (Gattani and Kaul 1951; Sherf 1952). Botrytis is a frequent pathogen of conifers and often colonizes many different types of seed, including those of forest trees (Coley-Smith et al. 1980; Neergaard 1977). Diplodia causes tip blight of pine and infects cones (Peterson 1981); it has been isolated from the seed of Austrian pine and several other plants (Neergaard 1977; USDA Forest Service 1948). Verticillium includes several species of common soilborne pathogens which usually cause wilt diseases (Hepting 1971). These fungi may also infect seed of many plants (Neergaard 1977).

Most of the other fungi isolated from ponderosa pine seed in our evaluation are probably saprophytes, although a few may be weak pathogens under certain circumstances. For example, although Trichothecium roseum is a common soil inhabitant often found on dead organic matter (Ingold 1956; Rifari and Cooke 1966), it may also cause decay of forest tree seed, including pine and spruce (Urosevic 1961). Gliocladium, another soil inhabitant similar to Penicillium (Smalley and Hansen 1957), and Chaetomium both may colonize seed (Neergaard 1977) and are antagonistic toward common pathogenic fungi (Hashioka and Fukita 1969; Malone and Muskett 1964; Weindling and Fawcett 1936). Ulocladium, Pyrenochaeta, Aspergillus, and Cephalosporium are common colonizers of organic debris, but may also infect seed of certain crops (Groves and Skolko 1944; Neergaard 1977; Raper and Fennell 1965; Reddy and Holbert 1924). Lacellina graminicola is common on dead organic matter, leaves, and stems (Barnett and Hunter 1972). Species of bacteria and yeasts were not determined and their effects on seed are unknown.

Potential of many of the fungi we found on pine seed to cause disease of seed and young hypocotyls is unknown. Pathogenicity tests are needed to help define disease potential of these fungi. We suspect that such tests might reveal that several of these organisms can cause seed deterioration and reduced germination.

Fortunately, the most probable pathogens we isolated from pine seed (Fusarium, Pythium, Botrytis, Verticillium) were not as common as other fungi. However, they did occur in sufficient numbers to cause concern. If infected seed is planted in previously sterilized seedbeds, losses may be high because of reduced populations of competing organisms. Since potential pathogens occurred within seedlots from all sources, we suspect that these fungi are common seed contaminants. Seed treatment with an effective sterilant will reduce external fungal contamination; care should be taken during treatment not to damage seed sufficiently to reduce germination. Unfortunately, many of the seed tested also contained fungi within the seedcoat. Therefore, some losses from decayed seed or damping-off may occur despite seed treatment.

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